Research Plan Proposal

Estimation of Biochemical Components of Ivy gourd*(Coccinia grandis)* and Its Effect on Blood Sugar level of type 2 Diabetics

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1. INTRODUCTION

Indian system of traditional knowledge *i.e.Ayurveda* is well known for its effective herbal treatment. There are about 7000 plant found in India. Although most of them have a long history in folk medicine, there is lack of written data on their efficacy, composition and medicinal properties especially from human studies. Many of them are used to treat highly prevalent disorder like Diabetes mellitus (Pathwardhan *et al*., 2005).

Recently, the world health organisation estimated that 80 per cent of people worldwide rely on herbal medicines for some part of their primary health care. In the past 20 years in United States, public dissatisfaction with the cost of prescription medication, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use (Ekor *et al*. 2014).

Plants have always been an exemplary source of drugs and many drugs currently available have been derived directly or indirectly from them. A vast majority of population particularly those living in villages depend largely on medicinal plants for treating and curing diseases. One such medicinal plant is, Ivy gourd (*Coccinia grandis*) is indigenous herb plant of Central Africa and Asia. It is cultivated abundantly in India (Assam, Bihar Orissa, Maharashtra, Rajasthan,Andhra Pradesh, and Tamil Nadu) as a vegetable and its wild form is also found in many parts of India. This plant has been widely used in traditional Indian medicinal system Ayurveda, Unani andSiddha (Yadav *et al*., 2010).

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use derived from medicinal plants, mineral and organic matters (Grover et al., 2002).

Ivy gourdherb plant has anti-diabetic activity and its different parts *viz.* Root, stem, leaf and fruit have been reported to show anti-diabetic effect in many previous studies. The herb contains good amount of Triterpenoids, Pectin and enzyme Glucose-6-phosphatase, which help in lowering blood sugar level (Kamble *et al*., 1998).

1.1 PLANT Ivy gourd *(Coccinia grandis*)

Ivy gourd*,* commonly known as baby watermelon is another tropical perennial creeper grown in India. It belongs to the family of cucerbitaceae and its scientific names are *Ivy gourd and Coccinia cordifolia.* ItsBinomial name is*Coccinis grandis* (L.) Voigt. A detail description of different parts of the plant is given below -

1.1.1 ***Leaves* -** Leaves are arranged alternately along the stems; the shape of the leaves varies from heart to pentagon and is up to 10 cm wide and long. The upper surface of the leaf is hairless, whereas the lower is hairy. There are 3–8 glands on the blade near the leaf stalk. Tendrils are simple (Imbuni, 2015).

1.1.2 ***Flower*-** Flowersare large, white and star-shaped. The calyx has five subulate, recurved lobes, each 2–5 mm long on the hypanthium and peduncle is 1–5 cm long. The corolla is white, campanulate, 3–4.5 cm long, deeply divided into five ovate lobes. Each flower has three stamens (Kumar *et al.,* 2018).

1.1.3 ***Fruit*-** The fruit is red, ovoid to elliptical, 25–60 mm long, 15– 35 mm in diameter, glabrous, hairless on stalks (Imbuni, 2015).

1.1.4 ***Seeds* –**The seeds*are* 6-7 mm long, tan-coloured and the margins are thickened (Imbuni, 2015).

1.1.5 Root- The roots and stems are succulent, tuberous and most likely facilitate the plant to survive prolonged drought (Imbuni, 2015).

1.2 Taxonomical Classification - Taxonomical classification of Ivy gourd*(Coccinia grandis*) is given in Table 1.1.

Table. 1.1: Taxonomical classification of Ivy gourd*(Coccinia grandis)*

Kingdom	**Plantae**
Clade	Angiosperm
Order	Eudicot
Family	Cucurbitaceae
Genus	Coccinia
Species	grandis

(Kumar ***et al***., 2018)

1.3Cultivation

Worldwide

- The range of native variety of *Ivy gourd*extent from Asia to Africa.It is produced inIndia, Malaysia, Srilanka, Thailand and Australia.

In India

- In India, the states in which Ivy gourd is cultivated are Assam, Bihar Orissa, Maharashtra, Rajasthan,Andhra Pradesh and Tamil Nadu. It is also known as*Tindora, Tondli,Tindora, Kundru and Kundri*in other languages.

(Kumar *et al*., 2018)

1.4 Nutrient composition of Ivy gourd *(Coccinia grandis*) –The nutrient content (macro and micronutrients) of fruit has been estimated by various scientists. These findings have been summarized in the Table 1.2 given below -

Table 1.2: Nutrient content of Ivy gourd *(Coccinia grandis)*

Nutrient	Rubatzky, *et al.*(1997)	Khatun *et al.* (2012)	Gautam *et al.*(2016)	ICMR (2017)	Alagarraja *et al*. (2018)
Energy	18 kcal	18 kcal	21 kcal	21 kcal	18kcal
Carbohydrate	3.1 g	3 g	3.4g	3 g	3.1g
Fat	0.1 g	0.2 g	0.2 g	0	0.1g
Protein	1.2 g	1.2 g	1.4g	1 g	1.2g
Calcium	40 mg	30 mg	25mg	37.12 mg	40mg
Iron	1.4 mg	2.2 mg	0.9mg	0.29 mg	1.4mg
Niacin	0.7 mg	0.7 mg	0.6	0.51 mg	0.7mg
Ascorbic Acid	1.4 mg	1.2 mg	1.6 mg	1.4 mg	1.4mg

1.2 NON COMMUNICABLE DISEASES

Non communicable disease also known as chronic diseases tend to be of long duration and are the result of a combination of genetic, physiological, environmental and behavioural factors. The main types of NCDs are cardiovascular, chronic respiratory disease, cancer and diabetes. NCDs contribute to around 68 per centof all death globally and to about60 millionof all deaths in India (WHO, 2018).

Due to sudden and drastic change inlifestyle of people and the environment, NCDs are found to be the most common cause of death (Global status of non-communicable diseases, 2010).According toWHO (2010) more than half of the deaths in the world are due to NCDs. Among these diseases,Diabetes mellitus is considered as one of the largest emerging threats to health in the 21st century

The term Diabetes mellitus describes a metabolic disorder of multiple aetiology that is characterized by chronic hyperglycaemia, due to disturbances in carbohydrate, fat and protein metabolism, which results from defects in insulin secretion, insulin action or both. Diabetes is a condition primarily defined by the level of hyperglycaemia giving rise to the risk of micro vascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to specific diabetes related micro vascular complications, increased risk of macro vascular complications (ischemic heart disease, stroke and peripheral vascular disease) and diminished quality of life. Diabetes mellitus is of two types – insulin dependent (type 1) and non-insulin dependent (type 2). An estimated 422 million adults in world are living with diabetes mellitus (WHO, 2016)

Indian Diabetes Federation (2019) reported that India stands second with 77 million people with diabetes. Apart from this there are many people who are yet to be diagnosed. Worldwide prevalence of Diabetes mellitus was 285 million in 2009 and it is estimated to increase to 438 million by 2025 and 700 million by 2045.

The increase in incidence of Diabetes mellitus in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly ‘a western style’ diet. Changes in life style such as increase in energy intake and decrease in physical activity are causing overweight and obesity and leading to an epidemic increase in Type 2 Diabetes mellitus and heart diseases (Barclay, 2008).

According to Roglic *et al.,* (2004) Diabetes mellitus, though, occurs throughout the world, but is more in the developed countries. According to the WHO (2000) at least 171 million people worldwide suffer from Diabetes or 2.8 percent of the population. The greatest increase in prevalence is however, expected to occur in Asia and Africa.

So there is an immediate need for some other alternative to control diabetes with lesser or no side effect. In the present research Ivy gourd, herb plant will be studied for its nutrient content and its impact on lowering blood glucose level among diabetic patients.

1.3 MEDICINAL VALUE OF HERB

Diabetes constitutes more than 15 percent population of our country. Modern allopathic system and medicine has not been able to reach the remote rural areas for various reasons. Large number of people can't afford the costly treatment and in recent years there is more inclination towards alternative therapies of treatment. Majorly treatment of diabetes involves use of drugs like sulfonylures, D-phenlaline, biguanides and alpha-glucosidase inhibitors with combination of insulin doses. As these drugs and insulin have some major side effects and are high in cost, there is an immediate need for shift on herbal treatment of diabetes with lesser or no side effect.

Many previous studies have supported the fact that Ivy gourd *(Coccinia grandis)* can be used in controlling diabetes. As reported, triterpenes can inhibit enzymes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insulin levels (Nazaruk et al., 2015). With regard to pectin, it was reported that when compared to other fibres, pectin is more effective in lowering blood glucose level (Weinstock and Levine, 1988). Also, pectin's ability to increase viscosity leads to lesser intestinal absorption of glucose (Kim, 2005). Enzyme glucose-6-phosphatase is required for conversion of glucose-6-phosphate to glucose in gluconeogenesis process. Ivy gourd herb inhibits enzyme glucose-6-phosphatase so no further formation of glucose occurs in the body (Kamble et al., 1998).

SIGNIFICANCE OF STUDY

Ivy gourd *(Coccinia grandis)* herb is popularly becoming famous as a diabetic herb. There are researches that show blood sugar lowering effect of leaf, fruit, stem and root of Ivy gourd in animals. However, very few researches have been conducted on humans as a subject, so far. Whatever, limited data is available; it reveals that the herb contains good amount of triterpenoids, pectin and enzyme glucose-6-phosphatase, which helps in lowering of blood sugar level.

Taking into consideration limited secondary data related to nutrient content of different parts of the herb plant and almost negligible availability of food products having Ivy gourd in the market, the present study has been designed, to firstly estimate various macro and micronutrients, antioxidants and enzyme glucose-6-phosphatsase content in different parts of the herb plant, *viz.* leaf, fruit, stem and root and then develop a product by incorporating the herb powder. Further,

an effort will be made to study the effect of Ivy gourd*(Coccinia grandis)* on blood sugar level of Type 2 diabetics.

1.5 OBJECTIVES

1 .To estimate biochemical components (Triterpenes, pectin and enzyme glucose-6-phosphatase) of different parts of Ivy gourd ((*Coccinia grandis)* viz. leaf, stem, fruit and root.

2. To study shelf life of Ivy gourd powder.

3. To develop food products, using Ivy gourd powder.

4. To find out effect of Ivy gourd powder on blood sugar among Type 2 diabetics.

2. REVIEW OF LITERATURE

The secondary data collected on nutritive value of Ivy gourd*(Coccinia grandis)* herb plant, its medicinal value, its glucose lowering effect among diabetics and developed products have been presented under the following heads -

2.1 Nutritive and Medicinal value of Ivy gourd *(Coccinia grandis)*
2.2 Prevalence of type-2 Diabetes mellitus
2.3 Impact of Ivy gourd *(Coccinia grandis)*herb on blood glucose level of diabetics
2.4 Product development usingIvy gourd *(Coccinia grandis)*

2.1Nutritive and Medicinal valueof Ivy gourd (*Coccinia grandis)*

Khatun *et al.* (2012) analysed chemical composition of Ivy gourd. The phytochemical screening showed methanolic extract of leaf to contain nutrients such as carbohydrates (3g), fat (0.2 g), protein (1.2g), niacin (0.7mg) and ascorbic acid (1.2mg). Minerals like potassium, phosphorous, sodium, iron and calcium were 3.38, 1.15, 0.95, 2.23 and 3.79 mg/100g, respectively.

Shah *et al.* (2010) had undertaken a study related to phytopharmacological chemistry of *Ivy gourd* herb and concluded that Ivy gourd fruit contains vitamin B, D, G and H. The leaves

contain vitamin B, D, and traces of E. They also reported anti hyperlipidemic, analgesic and anti-inflammatory and diuretic effect of the fruit.

The extract of Ivy gourd was reported to contain alkaloids, glycosides, tannin, saponins, flavonoids and other phyto-constituents such as cephalandrol, lupeol, sigma-7-en-3one, taraxerone and taraxerol (Sutar, 2010).

Deokate*et al*. (2012) studied phyto-constituents of Ivy gourd plant and revealed that aerial part has Heptacosane, Cephalandrol, β -sitosterol, Alkaloids Cephalandrins A and B. The fruit has β-Amyrin Acetate, Lupeol, Cucurbitacin B, Taraxerone, Taraxerol, βcarotene, Lycopene, Cryptoxanthin, Xyloglucan, Carotenoids, β-sitosterol, Stigma-7-en-3one. The root of the plant has Alkaloids, Starch, Fatty Acids, Carbonic acid, Triterpenoid, Saponin Coccinoside, Flavonoid, Glycoside, Lupeol, β-amyrin, β-sitosterol, Taraxerol.

Deokar and Kahadababadi (2012) carried out their work related to pharmacology and phytochemistry of *Ivy gourd.* The findings reported by researchers revealed thatthe herb possesses hypoglycemic, antidiabetic, hypolipidemic, hepatoprotective, larvvicidal, anti-inflammatory, analgesic and antipyretic activities. Various phyto-constituents, which were reported to be present in *Ivy gourd* were cephalandrol, tritriacontane, lupeol, b-sitosterol, cephalandrine A, cephalandrine B, stigma-7-en-3-one, taraxerone and taraxerol and concluded that terpenoids are responsible for antidiabetic activity.

A study was carried out by Hussain *et al*. (2011)on anatomical and physicochemical features of Ivy gourd and the results revealed presence of tannins, steroids, flavonoids, glycosides, saponin, alkaloids, proteins, amino acids and acidic compounds.

Tamilselvan *et al.* (2011) in their review projected presence of steroids in petroleum leaf extract of Ivy gourd and detected the compounds like tannins, glycoside, proteins, amino acid, saponins and alkaloids.

Biochemical investigation of *Coccinia indica* was conducted by Chandria *et.al*. (2010).The phytochemical screening of the 50% methanolic extract obtained from all the parts of *Coccinia indica* revealed that it contains carbohydrates, glycosides, fix oils and fats, proteins and amino acids, saponins, tannins, phytosterol, alkaloids, phenolic compounds, flavonoids.

A study conducted by Sarian *et al*. (2017) stated that flavonoids are one of the most important groups of bioactive compounds among secondary metabolites. The total number and configuration of hydroxyl groups existing on the compounds increased the antioxidant and antidiabetic effects of flavonoids.

Testa *et al*. (2016) in a review article has talked about possible role of flavonoids in the prevention of diabetic complications and reported that flavonoids may play role in many metabolic processes. The reviews revealed that flavonoids can modulate carbohydrate and lipid metabolism, attenuate hyperglycaemia, dyslipidaemia and insulin resistance, improve adipose tissue metabolism and alleviate oxidative stress and stress-sensitive signalling pathway and inflammatory processes.

Nazaruk*et al. (*2015) stated that triterpenes play an important role in the management of diabetes mellitus. Triterpenes can inhibit enzymes involved in glucose metabolism, prevent the development of insulin resistance and normalize plasma glucose and insulin levels. They have strong antioxidant activity and inhibit the formation of advanced glycation end products, implicated in the pathogenesis of diabetic nephropathy, embryopathy, neuropathy impaired wound healing.

A study conducted by Panda*et al*. (2017) on effect of alpha lipoic acid (APA) in treatment of type 2 diabetes. A total of 35 patients with diabetes were selected randomly who were under insulin therapy. Patients were supplemented with APA (300mg/day) for 6 months continuously and the result revealed that there was significant reduction in fasting blood glucose level from 161 to 122mg/dl.

Kooti *et al*. (2016) carried out a systematic review on role of medicinal plants in the treatment of diabetes and concluded that diabetes is mainly due to oxidative stress and increase in reactive

oxygen species that can have major effects. Many plants containing natural antioxidant like alkaloids help in maintaining blood sugar level as they improve the performance of pancreatic tissues by increasing the insulin secretion, increasing glucose absorption by muscle and fat tissues, prevention of glucose absorption from the intestine.

Okonta and Aguwa (2007) studied hypoglycemic activity of glycosides in diabetic rats. The rats were fed with 250mg/kg of glycoside extract for 10 days. Blood glucose level was reduced to 66.85±6.51 on 10^{th} day, which was 393.00±12.54 on day 1. From the study researcher reported that glycoside had blood sugar lowering activity.

Ramu *et al*. (2016) carried out a study on effect of beta–sitosterol on glycaemic status and glucose metabolism in alloxan induced diabetic rats. Diabetic rats were fed with beta–sitosterol (100mg/kg b.wt/day) for 28 days, as a result enzymeshexokinase, glucose-6-phospatase, fructose-1,6-biphosphatase and lactose dehydrogenase were reverted to normal levels by the administration of beta–sitosterol, and beta cells of pancreas regenerated to produce insulin, as a result insulin level improved in blood which positively promoted the activities of glucose utilisation.

2.2 Prevalence of type2 Diabetes mellitus

2.2.1 *Worldwide*

According to the diabetes atlas published by the International Diabetes Federation (2019), the number of people with type2 diabetes in the world is currently about 463 million and is expected to increase 700 million by the year 2045.The greatest number of people with diabetes was in between 40 and 59 years of age. One in two (232 million) people with diabetes goes undiagnosed and deaths estimated due to Diabetes is about 4.2 million.

According to Centre for Disease Control and Prevention (CDC), over 30 million people in United States have diabetes. The CDC also noted that 90 to 95% of cases involve type 2 diabetes. Middle aged and older adults are still at the highest risk for developing type 2 diabetes.

According to the CDC's (2017) National Diabetes Statistics report, adult aged 45 to 64 years were the most diagnosed age group for diabetes.

According to the report on Global Statistics on Diabetes (2015), globally diabetes hits particularly 'middle aged' people between 40 and 59 years of age, which cause serious s economic and social implications.

Jayendran et al. (2003) stated that in United States of America, diabetes is approaching as an epidemic proposition. It is estimated that in the year 2002 over 16 million Americans had Diabetes mellitus, in which 90% suffered from type 2 diabetes. Diabetes is the 7th most prevalent cause of death in the United States of America. In the study researcher showed concern about growing cases of type 2 diabetes and concluded that there is immediate need for action to decrease the rate of type 2 diabetes.

A study conducted by Yen et al. (2003) on the prevalence of type-2 diabetes in southern part of Iran. In study the prevalence of diabetes and glucose tolerance was found to be 13.6 per cent and 15.6 per cent, respectively. Prevalence for both genders was calculated as 23.1 per cent (10.2 for men, 12.9 per cent for women). From the study researcher concluded that diabetes became more prevalent as the age increased in people. The percentage of subjects with impaired glucose tolerance, in the age range of 30-39 years, was 8.3 per cent and in those of age 50 to 64 years was. 24.8 per cent.

2.2.2 *In India*

A study was conducted to assess the dietary pattern and lifestyle of diabetic patients in India by Shrivastava *et al.* (2014). A total 60 diabetic patients were selected, and from the study it was concluded that sedentary lifestyle and obesity are major reasons behind growing cases of diabetes. It was also concluded that age group of 45-65 years had maximum number of diabetic patients.

World Health Organization (2008) stated that 19.4 million individuals were affected by type2 Diabetes mellitus in India in the year 1995. It will increases to 57.2 million by the year 2025. It

states that every fifth diabetic patient in the world is in India and every fifth adult is in urban area. The global number of people with diabetes was 220 million in 2010 and reaching 300 million by the year 2025.

Makol *et al*. (2008) stated that in 1970, the prevalence of type2 diabetes among urban Indian population was 2.1 per cent, which had risen to 12-16 percent in 2008. Region-wise prevalence showed a higher rate in southern part of India. The prevalence was Hyderabad was 16.6 per cent, followed by Chennai (13.5) and Bangalore (12.4per cent). In eastern India, prevalence of diabetes in Kolkata was found to be 11.7 per cent and in Northern India, it was 11.6 per cent in New Delhi, whereas in Western India in Mumbai, it was 9.3 per cent. It is clear that in past two decades there has been a marked increase in the prevalence of type2 Diabetes mellitus among urban Indians. In rural India, the prevalence of diabetes is much lower than in urban population. Prevalence of diabetes in children is also increasing because of the effects of globalization in all the societies.

India leads the world with largest number of type2 Diabetes mellitus patients and is called as the 'Diabetic capital of the world' (Mohan *et al.,* 2008).

A survey was conducted in rural area of Nagpur district to determine the prevalence of abnormal glucose tolerance and to study itsassociation with various factors. The results showed that there was high prevalence of diabetes (3.67%) in rural area of Nagpur district (Kokiwar *et al.*, 2007).

Ramachandran *et al*. (2001) reviewed National Urban Diabetes studied prevalence by using a stratified random sampling method. A total of 11,215 subjects (5288 men and 5798 women) aged 20 years or above representing all socio-economic status categories, were examined, using Oral Glucose tolerance Test. The prevalence of diabetes and impaired glucose tolerance were 12.1 per cent and 14 per cent, respectively, with no gender difference.

2.3 Impact of Ivy gourd (*Coccinia grandis)* herb on blood glucose level of diabetics

The researches have shown glucose lowering effect of *Ivy gourd* herb in animals and humans. Different parts of the herb have also been further studied and the results are summarized in the paragraphs below.

2.3.1 *Leaves*

Ocvirk *et al*. (2013) studied the nutritive and medical properties of *Ivy gourd*leaves among individuals residing in rural and urban areas of Dhaka (Bangladesh). Sixty three randomly chosen individuals (health professionals, diabetic patients) were interviewed in a structured manner about their administration or use of plants for treating diabetes. One third of all informants were taking *Ivy gourd* as an anti-diabetic herb and showed mark improvement in blood glucose levels,which concluded that leaves oftraditional medicinal plant *Ivy gourd*(ivy gourd) has significant impact on improving blood glucose level.

Ghosh and Roy (2013) conducted a study to evaluate anti diabetic potential of methanolic extract of ivy gourd (*Coccinia indica)* leaves in streptozotocin induced diabetic rats. Hyperglycaemia was induced in overnight fasted rats by a single injection of 65mg/kg of streptozotocin. About 1 kg fresh leaves of Ivy gourd were dried in incubator for 2 days at 40°C and crushed in a grinder to make powder. Eighteen rats were divided in group of 3. Group I was kept as control group, group II and group III were given methanol extract of herb leaves at a concentration of 150 and 300mg/kg by oral route. Blood samples were withdrawn from tail vein of each animal and with the help of glucometer blood glucose levels were estimated. It was observed that there was a significant improvement in blood glucose levels of rats of group II and III, the blood glucose level decreased by 26 per cent and 27 per cent, respectively. Thus, the researcher concluded that leaves of herb have blood sugar lowering property.

Whitaker (2011) conducted a study to find out effectiveness of Ivy gourd (*Coccinia indica*) in reduction of blood glucose level among 80 diabetics in Rajasthan. A total 60 newly diagnosed type2 diabetic subjects (not on insulin therapy for glucose control) received 1000 mg of Ivy gourd (*Coccinia indica*) leave powder for a period of 28 days. Result showed that those taking

Ivy gourd leave powder experienced better blood sugar control, with a mean score of pre-test fasting blood glucose level as 145.5mg/dl on day 1 and mean score of post-test fasting blood glucose level as 119mg/dl on day 28. The data shows that there is a significant reduction of blood glucose level after the administration of Ivy gourd (*Coccinia indica*) leaves powder.

Sutradhar *et al.* (2011) carried out a study to evaluate the anti-hyperglycaemic effects of crude methanol extract of leaves in Swiss albino mice. The methanol extract of the leaf demonstrated significant anti-hyperglycemic activity, when administered to mice at doses of 50, 100, 200 and 400 mg extract per kg body weight. The highest level of serum glucose reduction was observed with an extract dose of 400 mg per kg body weight, in which serum glucose level was found to be reduced by 56.3 per cent. In comparison to this when standard drug, glibenclamide, was administered at a dose of 10 mg per kg body weight, reduction in serum glucose level in mice was observed as 55.5 per cent. The researcher concluded that the leaf extracts has considerable anti hyperglycaemic properties.

Jose and Usha (2010) carried out a study to assess the hypoglycaemic, hypolipidemic and antioxidant activities of *Ivy gourd*. The study was conducted on albino rats and the chemical, alloxan, was used for experimentally inducing diabetes among them. Thirty two adult albino rats were divided into four groups of 8 each. Group I served as normal control and group II was made as diabetic control. Group III and IV were made diabetic and administered orally with 200mg/kg of ethanolic extract of *Ivy gourd* leaves and 0.25 mg/kg of glibenclamide, respectively, for 45 days. There was a significant ($p<0.05$) reduction in blood glucose, serum cholesterol, triglyceride and lipid peroxides levels in rats treated with *Ivy gourd* as compared with the diabetic control. The results showed that the ethanolic extract of *Ivy gourd* leaves possessed significant hypoglycaemic, hypolipidemic and antioxidant effects.

Sutar *et al.* (2010) carried out a study on *Ivy gourd* leaves to observe anti diabetic activity of the herb. Oral administration of the ethanolic extract of *Ivy gourd* leaves, 200 mg/kg of body weight, were given to diabetic rats for 45 days, which showed decreased concentrations of blood glucose, lipids and fatty acids, palmitic, stearic, and oleic acid, whereas, linolenic and arachidonic acid and plasma insulin were elevated.

A study was carried out on leaf extract of *Ivy gourd.* This study was a double blind, placebo controlled; randomised trial. A total 60 type 2 diabetic patients aged between 35 and 65 were selected as sample, having fasting blood glucose level from 110 to180 mg/dl. Patients were treated with 500mg capsule made from *Ivy gourd* leaf extract for 90 days, during which subjects reported weekly to clinic to check the impact of administration of *Ivy gourd* leaf extract tablets on fasting blood glucose level. From the study, researcher revealed that there was mark improvement in blood glucose level of patients before and after administration of tablets made from leaf extract of *Ivy gourd* and concluded that *Ivy gourd* herb plant is useful in controlling blood sugar level among diabetics (Kurpad et al., 2008).

Doss and Dhanaba (2008) studied the anti-diabetic effects of aqueous extracts of leaves of *Ivy gourd* on normal and alloxan induced diabetic rats. The rats were divided into two sets; diabetic and non-diabetic. Group I as normal control, Group II consisted of alloxaninduced rats and served as diabetic control. Group III consisted of alloxan induced rats receiving Gliben clamide (synthetic antidiabetic drug) at 0.5 mg/kg body weight once a day orally for 10 days. Group IV consisted of alloxan-induced rats receiving *Coccinia grandis* (1 ml) once a day orally for 10 days. Group V consisted of normal rats receiving *Coccinia grandis* (1 ml) once a day orally for 10 days. Blood samples were collected through the tail before and after 10 days of drug administration. The blood glucose, urea, cholesterol, serum glutamate oxygenate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were determined for all the samples. The extracts of Ivy gourd produced significant changes in the alloxan-induced diabetic rats. The aqueous extracts of Ivy gourd reduced the glucose levels considerably. Treatment of the diabetic rats with Glibenclamide (10 mg/kg) also reduced blood glucose level. The prolonged treatment of Ivy gourd extracts on alloxan induced diabetes rats produced consistent reduction in the blood glucose levels. The aqueous extract also reduced urea, protein and cholesterol levels.

The crude leaf extract was studied by Manjula *et al.* (2007) to determine the hypolipidemic effect in alloxan induced diabetic rats and its effect to stimulate insulin level in the blood. Leaf extract of Ivy gourd alter favourably the activities of the regulatory enzymes of glycolysis and gluconeogenesis. The study revealed effect of Ivy gourd leaf extract not only ashaving

hypoglycaemic effect but also hypolipidemic effect. Further, the findings showed that administration of Ivy gourd leaf extract also prevented elevation of the levels of serum lipids secondary to the diabetes state.

Jeannete and Goguen (2005) conducted a study on alternative medicine for glycaemia control in type2 Diabetes mellitus patients at Toronto. One randomized controlled trial studied 32 patients with type2 Diabetes for 6 weeks; 10 out of the 16 participants who received the experimental preparation of Ivy gourd (*Coccinia grandis*) (1.8 g/day) showed an improved glucose tolerance, while none of the 16 patients in the placebo group showed an improvement ($p<0.001$). On an average, fasting blood glucose level decreased by 2.4 mmol in the experimental group.

Shibib*et al*. (1993) reported that oral feeding of ethanol extract of Ivy gourd leaves (200 mg/kg) to 18 hour fasted rats and streptozotocin diabetic rats led to lowering of blood sugar by 23 per cent and 27 per cent, respectively, hepatic glucose-6-phosphatase by 19 per cent and 32 per cent, respectively hepatic fructose1,6-bisphosphatase by 20 per cent and 30 per cent, respectively, as compared to control group.

Kamble*et al*. (1998) carried out a clinical study (n=30) in which oral administration of dried extract of Ivy gourd (500 mg/kg for 6 weeks) significantly restored the raised activity of lipoprotein lipase and the levels of G-6 phosphatase and LDH, which are otherwise increased in the severe diabetics. This action of the plant extract was similar to that of insulin.

Platel and Srinivasan (1997) reported that a single oral dose of plant extract has been shown to exert beneficial hypoglycaemic effect in experimental animals and human diabetic subject possibly through an insulin secreting effect or through influence of enzymes involved in glucose metabolism.

Khan*et al*. (1979) studied the beneficial effects of leaves of Ivy gourd have in a double-blind control trial enrolling 16 patients with uncontrolled maturity onset diabetes and 16 controls. Treatment was given for 6 weeks and 10 patients showed marked improvement in their glucose tolerance.

2.3.2 *Fruit and Root*

Packirisamy *et al.* (2018) conducted a research to assess anti diabetic activity of the mature unripe fruit of *Ivy gourd* in streptozotocin (STZ) induced diabetic rats and its effect on carbohydrate metabolizing enzymes. The effects of *Ivy gourd* extract (CGE) was also compared with glibenclamide (hypoglycaemic drug). Results revealed that oral administration of *Ivy gourd* extract 250 mg/kg body weight once daily to diabetic rats for the period of 30 days had significant reduction in the blood glucose levels. Administration of CGE showed decrease in the levels of gluconeogenic enzymes in the liver of diabetic treated rats, the anti-hyperglycaemic effect of the CGE was also found to be comparable with glibenclamide drug, and therefore the study concluded that the fruits of CGE showed marked improvement in glucose level of blood.

A research carried out by Harine *et al.* (2017) on fruit of Ivy gourd to study its nutrient composition, medical properties, antioxidant properties, anti-bacterial properties and its hypoglycaemic effect. The researchers reported that herb helps in lowering blood sugar, helps in regulating body temperature during fever, used as an injection into chronic sinuses, used to treat inflammation, dyspnea, cough, emaciation and fever with burning sensation.

Yadav *et al.* (2010) in the review article has talked about medical importance, chemical profile, antibacterial, anti-protozoal, antiulcer, hepaticprotective, expectorant, analgesic, anti-inflammatory and pharmacological actions of ivy gourd (*Coccinia grandis*).About effect of ivy gourd herb (*Coccinia grandis*) on diabetes, the researcher concluded that alcoholic extract of Ivy gourd leaves significantly depresses the glucose-6-phosphatase and fructose-1,6-bisphosphatase activities, which lead to blood sugar lowering in diabetic patients.

Medagana *et al.(* 2014) studied the hypoglycaemic effects of cinnamon, bitter gourd, fenugreek and ivy gourd (*Coccinia grandis*) on using them as complementary and alternate to medicine to treat hyperglycaemia. The researchers reported that pectin; isolated from the fruit of Ivy gourd has shown hypoglycaemic properties in rats. They also reported that it also possesses the ability to correct elevated levels of glucose-6phosphotase and lactase dehydrogenase and thereby correct hypoglycaemia.

Kumar *et al*. (2010) conducted a study to evaluate effectiveness of the pectin component in ivy gourd fruit to reduce the serum blood glucose level. The study showed administration of pectin isolated from the fruit of the Ivy gourd at a dose of 200 mg or 100mg/kg body weight/day showed a significant hypoglycaemic action in normal rats due to stimulation of glycogen synthetase activity.

A systematic review of the published literature on the efficacy and safety of herbal supplements for glucose control in patients with diabetes was conducted by Gloria *et al.* (2003). A total 36 herbs were chosen for the study. Fifty eight controlled clinical trials involving patients with diabetes or impaired blood glucose tolerance (42 randomized and 16 nonrandomized trials) were treated with ivy gourd fruit for a period of 30 days and the Meta analysis showed improved glucose control of in 76 per cent of diabetic patients.

Chandrasekar *et al.* (1989) carried out a study in which oral administration of ethanolic extract of *Ivy gourd* root (250 mg/kg) to normal rats significantly lowered blood sugar in fasting rat and depressed the peak value in glucose loaded model.

2.4Product development using Ivy gourd*(Coccinia grandis)*

Shigihalli *et al*. (2018) carried out a study to develop a jelly from ivy gourd (*Coccinia grandis*). Three variations of ivy gourd jelly containing different levels of ivy gourd powder (10g, 20g and 30g) were prepared. Among the variations 10 per cent level incorporation scored higher for overall acceptability. The best accepted variation and control jellies were filled in glass jars and were stored at refrigerated temperature up to 21 days. Evaluation of fresh as well as stored samples for changes in chemical, sensory, microbial and moisture attributes was done at an interval of 7, 15 and 21 days. From the study it was concluded that sensory scores were decreased with increased storage period for both the control and best accepted variation. However, pH and moisture content were observed to decrease with increase in storage period. Microbial population was also found to increase with increase in storage period. However, the

developed jelly was safe and suitable for consumption over the storage period. The findings revealed that one can use dehydrated ivy gourd for the development of jelly.

Vinothini *et al*. (2015) reported that incorporating ivy gourd fruit powder with pulses, many traditional fried snacks can be prepared. Snack food incorporated with 7g ivy gourd powder, fried at 190°C was evaluated for storage and nutrient content. The findings revealed nutrient content to be 5.3% protein, 85% carbohydrate, 23.2% of fat, 3% minerals, 0.104 % antioxidants, 1% moisture content and 0.023% vitamin A. The colour of the snack food was found to be reddish yellow. The shelf life study was performed with LDPE (low density polythene) and metallised polyester. There were not many changes when nutritional parameters were analysed for 0, 15, 30 and 45 days but there was increase in peroxide value from 37.75±0.012 to 40.63±0.002 meq/kg. Microbial analysis showed that more colonies were present in LDPE compared with metallised polyester. From the findings researchers concluded that metallised polyester is best suited for packing fried snack food incorporated with ivy gourd for a shelf life of 45 days.

3. METHODOLOGY

The present study will be carried out on herb commonly known as Ivy gourd (*Coccinia grandis*). Various parts of the herb plant, *viz.* leaf, fruit, stem, and root will be analysed for macro and micronutrients, antioxidants and enzyme glucose-6-phosphatase content. On the basis of biochemical analysis the herb powder will be provided to the diabetic subjects, either in combination or alone to assess its effect on blood glucose levels.

The present study will be carried out in 3 stages –

3.1 STAGE I – NUTRIENT ANALYSIS

This stage will include analysis of nutrients, antioxidants and enzyme glucose-6-phosphatase content indifferent parts of the Ivy gourd herb, *viz.* stem, leaf, fruit and root. These nutritional components will be analysed in triplicates, using standard techniques and analytical grade chemicals. Stage I will consist of following steps:

3.1.1 Step1 -Procurement of the herb stem and cultivation

The Ivy gourd *(Coccinia grandis)* herb will be procured from the market by the investigator.

3.1.2 Step 2 - Biochemical analysis of Ivy gourd (*Coccinia grandis*)

The selected four parts of the herb Ivy gourd (leaf, fruit, stem and root)will be plucked for analysis when the fruit of the plant will become dark green in colour with white streaks, fusiform ellipsoid, slightly beaked, 2.5-5cm by 1.3-1.5cm in size (Kumar et al., 2018) and yellow from inside (Imbumi, 2004).These will be then washed thoroughly with water and dried in shade for two weeks(Chandaka *et al*., 201). Once dried, the different parts of the herb Ivy gourd will be ground separately, using electric grinder and will be stored in air tight containers until in use for analysis (Chandaka *et al*., 2013).

- Leaf, fruit, stem and root of herb Ivy gourd will be analysed for macronutrients *viz.* moisture, protein, fat, fibre, ash and pectin. Carbohydrate will be determined using composite method and energy will be calculated. Various micronutrients that will be estimated are β carotene, vitamin B, vitamin C, vitamin E, potassium, phosphorous, and

calcium. Enzyme glucose-6-phosphatase will also be analysed. Nutrient analysis will be carried out in the Research laboratory of Department of Home Science, IISU.

- The Antioxidants *viz* .triterpenoid, alpha lipoic acid, betasitosterol, alkaloid, glycoside, N-actylcestene and total flavonoids will be analysed at an analytical laboratory located in Jaipur.
- For biochemical analysis, aliquots will be prepared from the samples of different parts of the herb plant and will be analysed in triplicates using standard technique and analytical grade chemicals. The methods that will be used to analyse nutritional components *viz.* macro and micronutrients, antioxidants and enzyme are given in Table 3.1, 3.2 and 3.3, respectively.

Table 3.1Methods to be used for estimation of macro and micronutrients of Ivy gourd*(Coccinia grandis)*herb

S.No.	Nutrient (g/100)	Method	Reference
1.	Moisture	Oven drying	AOAC,2017
2.	Protein	Micro-kjeldhal	AOAC,2017
3.	Fat	Ether extractive	AOAC,2017
4.	Fibre	Acid alkali treatment	AOAC,2017
5.	Ash	Muffle furnance	AOAC,2017
6.	Carbohydrate	Composite method	AOAC,2017
7.	Pectin	Chromatography	AOAC,2017
8.	Energy	Calculation	AOAC,2017
9.	β carotene	Spectrophotometry	NIN,2003
10.	Vitamin C	Tritration	AOAC,2017
11.	Vitamin E	Spectrophotometry	NIN,2003
12.	Calcium	Tritration	Rangana, 2010
13.	Potassium	Flame photometry	Rangana, 2010
14.	Phosphorous	Colorimetry	Rangana,2010

Table 3.2Methods to be used for estimation of antioxidant content of Ivy gourd*(Coccinia grandis*) herb

S.No.	Antioxidant	Method	Reference
1.	Triterpenoids	HPLC	Harborne (1998)
2.	Alpha lipoloic	HPLC	Mottaram *et al*.(1997)
4.	Flavonoids	Colorimetry	Bohm and Kocipai(1994)
5.	Beta sitosterol	HPLC	Mallick *et al*.(2014)
6.	Alkaloid	HPLC	Harborne (1998)
7.	Glycoside	HPLC	Harborne (1998)

Table 3.3 Methods to be used for estimation of enzyme glucose-6-phosphatase of Ivy gourd *(Coccinia grandis)*herb

S.No.	Enzyme	Method	Reference
1.	Glucose -6- phosphatase	Spectrophotometry	AOAC,2017

3.2 STAGE II – FOOD PRODUCTS DEVELOPMENT

Keeping in mind the aim of the present study that is to determine the effect of herb in lowering blood glucose levels of the diabetic subjects, 6 to 8 food products will be developed by incorporating dry powder of Ivy gourd *(Coccinia grandis).*

The basic recipes will be selected from an authentic source and will be standardized in the Foods and Nutrition laboratory, Department of Home Science, IISU. The basic recipes will be standardized for its various attributes *viz.* portion size, shape, colour, taste, *etc.* Ivy gourd herb (*Coccinia grandis)* will be added in standardised recipes either in combination or alone in different proportions. The developed food products will undergo sensory evaluation by a panel of 10 judges, selected on the basis of sensory threshold test. The developed food products will be

assessed for various sensory attributes, like appearance, colour, texture, taste, odour and overall acceptability, using a nine point hedonic scale.

3.2.1 Shelf life assessment of Ivy gourd (*Coccinia grandis*) powder

Shelf life assessment of powder of selected parts of the herb will be carried out using sensory, microbial and biochemical evaluation techniques, over a period of 3 months at an interval of 15 days.

a) **Sensory/organoleptic**: Product will be assessed for following sensory attributes –colour, appearance, odour, taste, texture by a panel of 10 semi trained judges to determine the acceptability
b) **Microbial**: For this Standard plate count method will be used.
c) **Biochemical**: Product will be assessed on the basis of its peroxide value, moisture content nutrient content (protein, fat, pectin), antioxidant content (Triterpenoid) and enzyme Glucose-6-Phosphtase.

3.3 STAGE III– EFFICACY ASSESSMENT OF IVY GOURD AMONG DIABETICS

Stage III aims to assess the efficacy of Ivy gourd (*Coccinia grandis)*powder, in lowering blood glucose level among type 2 diabetic adult males.

3.3.1 Locale of the study

The study will be conducted in Metro Hospital, Mansarovar, Jaipur

3.3.2 Sample selection

A total of 50 outdoor adult males suffering from type 2 Diabetes mellitus will be selected for the study, using purposive sampling technique.

3.3.2. i *Inclusion criteria*

- Subjects with type 2 diabetes.
- Subjects not suffering from any other non-communicable diseases.
- Age 45-65 years.
- Subjects on hypoglycaemic drugs.
- Fasting blood sugar level between 140-180 mg/dl.
- Diabetic male subjects willing to participate.
- Residents of Jaipur city.

3.3.2. ii *Exclusion criteria*

- Subjects on insulin therapy.
- Type 1 diabetic subjects**.**
- Subjects suffering from any other non-communicable disease.
- Fasting blood sugar level above 180 mg/dl.
- Subjects below 45 years and above65 years of age.
- Residents of other cities.

3.3.3Data Collection

A semi structured interview schedule will be used to collect information from the selected subjects. The interview schedule will have questions under the following heads-

3.3.3.i. *Demographic profile :*

Information regarding name, age, religion, caste, literacy level and occupation of the subject, family type, size and income.

3.3.3.ii. *Subject profile:*

Information related to duration of diabetes, treatment followed, frequency of check-ups, etiology, acute and chronic complications, diet related restrictions, alternate therapy, etc.

Secondary data will be referred for the amount of herb powder that can be incorporated to observe the effect on blood glucose level. The detail of the same is given below in table 3.4.

3.3.4. Study design

A study will be carried out to assess the efficacy of Ivy gourd powder on lowering blood glucose level as well as on HbA1C level of 50 Diabetic adult males, visiting Metro Hospital, Jaipur, over a period of three months.

For carrying out study, prior permission from the ethical committee of Metro Hospital, Mansarovar, Jaipur / IISU will be obtained. Also, consent of the subjects will be taken in a predesigned form; thereafter the study will start.

Study will be conducted to assess the effect of Ivy gourd in lowering blood glucose levels of Type 2 diabetic subjects (50). Before providing the Ivy gourd powder to Diabetics the investigator will train them about the dose, timing and how to consume the powder. The information about food products (6-8), developed by incorporating ivy gourd (*Coccinia grandis*) powder will also be provided to diabetics by sharing videos and recipes through personal interaction. Printed pamphlet of six to eight recipes will also be given to each diabetic.

Blood glucose level will be estimated at an interval of 30 days i.e. 1^{st}, 30^{th}, 60^{th} and 90^{th}day. Pre and post-test of blood sugar level of diabetics will be carried out to assess the pattern and efficacy of ivy gourd powder in lowering blood glucose levels of diabetics from day 1 to day 90. HbA1C level of blood glucose will also be evaluated on day 1 and day 90, to assess blood glucose lowering impact among subjects consuming Ivy gourd powder for 90 days.

3.3.5 Statistical Analysis

The collected data will be analysed using descriptive statistics (frequency percentage, mean and standard deviation).ANOVA test will be used to determine the difference between pre and post fasting blood glucose level as well as HbA1C level of the selected patients to assess the impact of Ivy gourd.

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